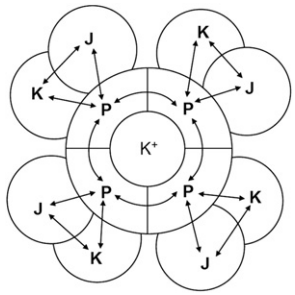
3149-Pos Board B254
Modular Allosterism in Potassium Channels
Daniel M. Sigg, Riccardo Olcese.
Allosteric interactions in ion channels serve to sharpen the control of environmental influences on conductance. We investigated the thermodynamic and kinetic properties of a multi-allosteric K^+ channel model possessing three distinct regulatory sites on each of four homologous subunits. Each site was characterized by a bistable free energy landscape with equilibrium constant *J,K*, or *P* that was sensitive to a particular environmental variable, as well the activation state of neighboring domains (see figure). The **P** domain comprising the central pore domain was defined by strong nearest-neighbor interactions that, combined with a counter-balancing bias in *P*, raised the energy and significantly shortening the dwell times of subconductance states. The mean number of activated regulatory sites was derived from the partition function through linkage analysis. A numbering scheme based on subunit occupancies of the 2^3 single-subunit activation states generated 330 energetically distinct kinetic states. Solutions to the rate equations simulated unique features attributed to different voltage-dependent K^+ channels (for example, intermediate pore activation states in the BK channel, and the rising phase and relationship with activation charge displacement observed in Shaker gating currents), supporting the feasibility of using a modular allosteric approach to K^+ channel activation.



3150-Pos Board B255
Voltage Sensor Deactivation Inhibits BK Channel Opening by Mg^{2+}
Ren-Shiang Chen, Yanyan Geng, Karl L. Magleby.
Functional and structural studies suggest that intracellular Mg^{2+} activates BK channels through interaction with the voltage-sensing domain (Yang et al. 2007, 2008; Horrigan & Ma 2008; Yuan et al. 2010; Wu et al. 2010). To further explore the mechanism of activation of BK channels by Mg^{2+} through the low affinity E374/E399 Mg^{2+} sites located beneath the voltage sensors, we use single-channel analysis to study BK channels mutated to remove the high affinity Ca^{2+} sites. We find that 10 mM Mg^{2+} shortens the latency to first channel opening after a voltage jump to +100 mV from −100 mV, consistent with the hypothesis that Mg^{2+} can bind to the closed channel and shorten the latency. However, it is not clear whether the closed-channel binding occurs when the voltage sensors are deactivated (down) or activated (up). We therefore recorded single-channel activity in macro-patches held at constant −50 mV, where voltage sensors occasionally activate. Under this condition, 10 mM Mg^{2+} decreases mean closed duration and increases mean open duration. These effects are attenuated at −100 mV, and become negligible at −150 mV, where the voltage sensors are mainly deactivated. For BK channels modified to have deactivated voltage-sensors (R167E), 10 mM Mg^{2+} has little effect on mean closed and open durations at −50 mV. In contrast, for BK channels modified to have constitutively activated voltage sensors (R210C), 10 mM Mg^{2+} shortens the mean closed durations and lengthens the mean open durations at −200 mV. The above observations are consistent with a model in which voltage sensor deactivation inhibits BK channel opening by Mg^{2+}. Supported by NIH grant AR32805 and AHA 10POST4490012.

3151-Pos Board B256
Influence of Hydrophobic Residues on BK Channel Gating
Guido Gessner, Toshinori Hoshi, Stefan H. Heinemann.
Large-conductance calcium- and voltage-activated potassium (Slo1 BK) channels participate in the control of vascular tone and neurotransmitter release. A neuronal splice variant of BK channels with altered sequence in the S6/RCK1 linker exhibits increased open probability in 100 μM calcium at −150 mV (PoC) compared to "wild-type" BK channels (Soom et al. 2008, Channels 2:278-282). To identify amino-acid residues underlying this notable change in gating behavior, we expressed wild-type and mutant human Slo1 BK channels in HEK 293 cells and analyzed their calcium- and voltage-dependent gating in the inside-out configuration of the patch-clamp technique. Mutation G327L, located in the linker connecting the S6 helix to RCK1, most strongly increased PoC from 0.02 to 0.24. Systematic substitution at that site revealed that hydrophobicity is most important for channel opening at low voltages. PoC for G327F and G327Y were 0.19 and 0.01, respectively; addition of a single hydroxyl group ("F327Y") decreased PoC by 0.18. The same change in hydrophobicity by mutation F315Y within S6 has qualitatively opposite effects (Lippiat et al. 2000, J Physiol 529:131-138). Phenylalanine scanning mutagenesis of the S6/RCK1-linker region revealed that the mutation K330F most strongly increased PoC to around 0.5. Introduction of an additional hydroxyl group at this site ("F330Y") decreased PoC by 0.37 down to 0.13. We conclude that hydrophobicity within the S6/RCK1-linker region is a critical determinant of the calcium-dependent gating.

3152-Pos Board B257
Voltage-Dependent Inactivation Gating at the Selectivity Filter of the MthK K+ channel
Andrew S. Thomson, Brad S. Rothberg.
Voltage-dependent K+ channels can undergo a gating process known as C-type inactivation, which involves entry into a non-conducting state through conformational changes near the channel's selectivity filter. Here we report on a form of voltage-dependent inactivation gating observed in MthK, a prokaryotic Ca2+ -gated K+ channel. In single-channel recordings, we observe that Po decreases with depolarization, with a half-maximal voltage of 96 ± 3 mV. This gating is kinetically distinct from blockade by internal Ca2+ or Ba2+, suggesting it may arise from an intrinsic inactivation mechanism. Inactivation gating was shifted toward more positive voltages by increasing external [K+] (47 mV per 10-fold increase in [K+]), suggesting that K+ binding at the extracellular side of the channel stabilizes the open-conductive state. The open-conductive state was stabilized by other external cations, and selectivity of the stabilizing site followed the sequence: K+ ≈ Rb+ > Cs+ > Na+ > Li+ ≈ NMG+. Selectivity of the stabilizing site is weaker than that of sites that determine permeability of these ions, suggesting that the site may lie toward the external end of the MthK selectivity filter. We could describe MthK gating over a wide range of positive voltages and external [K+] using kinetic schemes in which the open-conductive state is stabilized by K+ binding to a site that is not deep within the electric field, with the voltage-dependence of inactivation arising from both voltage-dependent K+ dissociation and transitions between non-conducting (inactivated) states. These results provide a quantitative working hypothesis for voltage-dependent, K+-sensitive inactivation gating, a property that may be common to other K+ channels.

3153-Pos Board B258
C. Elegans Slo-2b uses its RCK1 Domain as a Ca^{2+} Sensor and does not Exhibit Cl^- Dependence
Zhe Zhang, Qiong-Yao Tang, Diomedes E. Logothetis.
Slo-2 channels play an important role in the adaption of neuronal firing rates and have been implicated in protection against ischemia. Slo-2 channels belong to the family of high-conductance potassium channels but their gating mechanism is unique and has been reported to exhibit species differences. The rat Slo2 (Slack) channel is activated by Na^+ and Cl^-, whereas the *C.elegans* Slo-2a has been reported to be sensitive to Ca^{2+} and Cl^-. Here, we report isolation of a novel isoform of the *C. elegans* channel Slo-2b (F08b12.3c) that was cloned from ESTs (YK1522e1, YK1193) of *C.elegans*, which has a distinct N-terminal region (by 18 amino acids) compared to the previously reported Slo-2a (F08b12.3b) (Yuan et al, 2000). This new clone shows voltage- and Ca^{2+}-activated macroscopic currents when expressed in *Xenopus* oocytes. We find that the *C. elegans* Slo-2b channel isoform exhibits a unitary conductance consistent with Slo-2a but it is not activated by Cl^-. Furthermore, the current characteristics of Slo-2b can be described well by the Horrigan-Aldrich model, which had been developed to describe Slo1 current properties. Mutagenesis screening revealed that the Slo-2b channel with mutation of a critical Glu residue in the RCK1 domain largely controls Ca^{2+} sensitivity. In contrast, mutations of negatively charged residues around the region corresponding to the Na^+ sensitive site of Slack channels in the RCK2 domain do not affect Ca^{2+} sensitivity of the Slo-2b channel. Thus, we conclude that the Ca^{2+} sensor of the Slo-2b in the RCK1 domain is largely sufficient to confer Ca^{2+}-sensitivity to the Slo-2b channel isoform.

3154-Pos Board B259
An Epilepsy/Dyskinesia-Associated Mutation in BK Channel Enhanced Channel-PIP_2 Apparent Affinity
Qiong-Yao Tang, Zhe Zhang, Vasileios I. Petrou, Diomedes E Logothetis.
Large conductance, Ca^{2+}- and voltage-gated BK (Slo1) channels are critical for neuronal functions. A previous study has shown that BK channels are regulated by membrane phosphatidylinositol 4,5-bisphosphate (PIP_2). However, the mechanism of such regulation remains largely unknown. Here we show that an Asp-to-Gly mutation (D369G) associated with the human syndrome of generalized epilepsy and paroxysmal dyskinesia (GEPD) enhanced channel-PIP_2 sensitivity in BK channels. With 106 mM Ca^{2+} in the bath, the inside-out patch G-V curve of D369G was leftward shifted by ~ −28mV, suggesting enhanced channel activity for the mutated channel. Following depletion of endogenous PIP_2 by poly-lysine, PIP_2 activated D369G in a dose- and voltage-dependent manner. The EC_{50} for diC8-PIP_2 activation of D369G decreased ~4-fold compared to the WT channel, suggesting an enhanced channel-PIP_2 interaction. Structural models of the BK channel place D369 near the membrane, where it could interact directly with PIP_2. To study the mechanism of BK channel regulation by PIP_2, we also used neomycin, a polycation that binds PIP_2, as an indirect assay of channel-PIP_2 affinity. We tested whether the D362G and D367G